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Title of Grant: Multiscale Modeling of Blood Flow and Platelet Mediated Thrombosis

Abstract Authors

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Abstract Text

Introduction: Continuum-based methods fail to cover the vast spatio-temporal scales required to describe complex platelet events comprising flow-induced thrombosis. Our previously developed multiscale modeling (MSM) approach circumvents limitations of such methods by incorporating coarse-grained molecular dynamics (CGMD) and dissipative particle dynamics (DPD) to describe mechanotransduction events triggered by blood flow in cardiovascular pathologies which may induce initiation of thrombosis via flow-induced platelet activation[1-5]. This model, tightly coupled to extensive *in vitro* measurements of platelet motion under flow[1,2], mechanical properties[3,4], and shape change[5], has been expanded to describe early shear-induced platelet aggregation and adhesion.

Materials and Methods: Our CGMD-DPD model, which describes the nanoscale mechanotransduction and biophysics of deformable platelets under viscous blood flow[3], was separately adapted for recruitment aggregation and adhesion simulations of marginated platelets. Each platelet in the aggregation model has 67,004 GPIIb-IIIa receptors represented by the particles of the bilayer membrane (Fig. 1), while platelets in the adhesion model has 16,751 particles representing GPIbα receptors (Fig. 2). Our previous multiple time-stepping (MTS) scheme[6,7] was modified using event-driven adaptive time stepping (ATS) to adapt to platelet dynamics on top supercomputers, with computational efficiency achieved with supervised machine learning (ML) approaches. Simulated recruitment aggregation was validated in vitro, and parameters for the adhesion model were extracted from platelet adhesion experiments. Reconstituted whole blood (red blood cells and platelets) were perfused at shear stresses up to 30 dyne/cm^2 through vWF-coated microchannels to induce margination and adhesion, followed by perfusion of platelets with 1.5 mg/ml fibrinogen at shear stresses 1-10 dyne/cm^2. Platelet aggregation and adhesion events were captured with DIC microscopy (Nikon Ti-Eclipse with 100× magnification) at up to 1000 fps (Andor Zyla sCMOS camera). Platelet physical and geometric parameters (angular velocity, translational velocity, diameters, aspect ratio, circularity) were analyzed from captured images using Nikon NIS-Elements and ImageJ, and integrated three-dimensionally to determine surface and contact areas. Contact area between aggregating platelets were predicted by input of geometric parameters into a database to train a 2-layer, 10-node neural network (NN) machine learning-based aggregation model with Bayesian regularization.

Results and Discussion: Fig. 1 describes recruitment of marginated platelets and initiation of platelet-platelet aggregation. Binding of GPIIb-IIIa and fibrinogen during recruitment was mimicked using a molecular-level hybrid force field that combines modified Morse and Hooke potentials to reproduce morphologic characteristics as contact area at aggregation. We compared rigid and deformable platelets and observed that a rigid model significantly underestimated the contact area of aggregated platelets, as validated *in vitro*. In addition, numerically simulated GPIIb-IIIa – fibrinogen bond detachment forces were within the range of previously published experimental observations. Preliminary adhesion experiments show two distinct periods, horizontal to vertical (ω_1) and vertical to horizontal (ω_2), during platelet flipping on vWF-coated surfaces. At 6.7 dyne/cm^2, the duration of these periods is 16.35±5.14 ms and 13.62±4.16

ms, respectively, indicating the influence of GPIbα-vWF bond formation and breakage on flipping angular velocity (n=30, p>0.05). Ongoing experiments analyze this behavior in detail at shear stresses up to 30 dyne/cm^2 and frames rates of 1000 fps to validate parameters in the numerical model.

Conclusions: Our computationally affordable, highly resolved, and validated multiscale modeling approach provides a potentially predictive platform to describe shear-induced activation, aggregation, and adhesion in shear flow down to the nanoscales. Ongoing simulations and experiments currently evaluate aggregation events with multiple platelets and incorporate GPIbα-vWF interactions for adhesion at moderate to high shear stresses. Our validated models can be used to test development of new anti-platelet therapeutic approaches that modulate platelet membrane and other biophysical properties to make the platelet more shear resistant. We are utilizing MSM to analyze the impact of clinically relevant shear forces generated via a range of devices and pathologies to predict cellular responsiveness driving thrombosis.

In this multiscale model, the 10 simple rules of model credibility were addressed (see the attached Table).

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References: [1] Zhang, P., et al, *Cell Mol Bioeng*, 7:552-574, 2014. **[2]** Gao, C., et al, *J Comput Phys*, 335:812-827, 2017. **[3]** Zhang, P., et al, *J Biomech*, 50:26-33, 2017. **[4]** Zhang, N. et al, *J Comput Phys*, 257:726-736, 2014. **[5]** Pothapragada, S., et al, *Int J Numer Meth Biomed Engng*, 31:1-16, 2015. **[6]** Zhang, P. et al, *J Comput Phys*, 284:668-686, 2015. **[7]** Zhang, P. et al, *Comput Phys Commun,* 204:132-140, 2016.

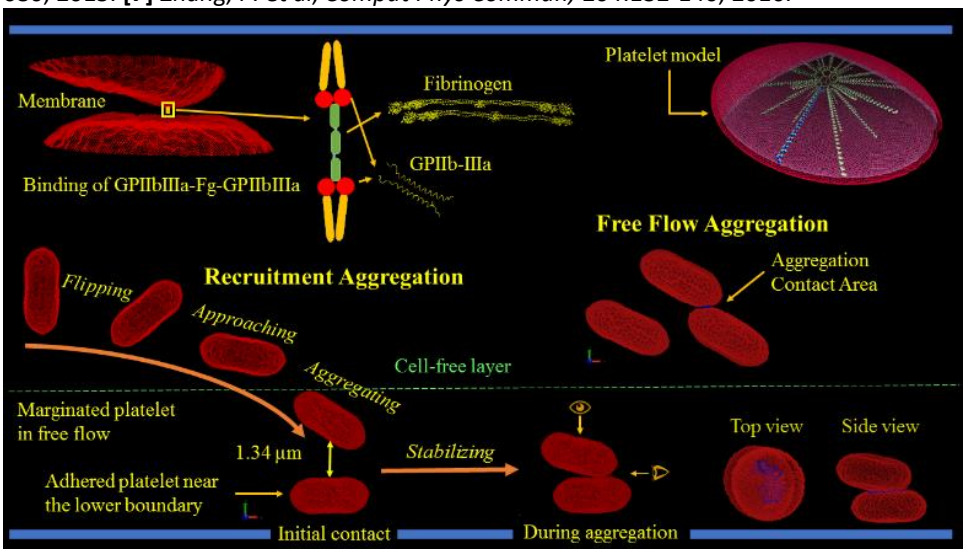

Fig. 1: Multiscale model of aggregating platelets

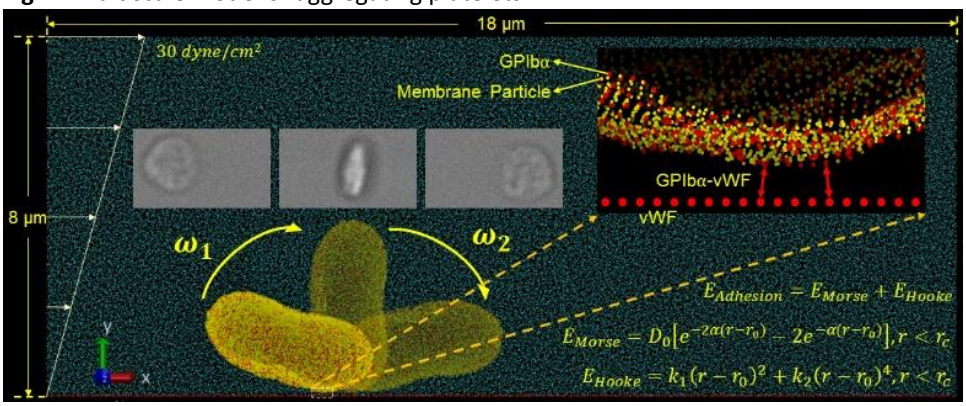

Fig. 2: Multiscale model of platelet adhesion.

Table: 10 Simple Rules of Model Credibility Gained

Rule 1. Define context clearly	Our DPD-CGMD models are designed to reflect platelet properties and dynamics under shear stresses found in blood flow through diseased vessels and cardiovascular devices.
Rule 2. Use appropriate data	We ensure that all parameters and input variables are based on published and in-house in vitro observations. If any parameters cannot be validated (due to lack of available data or techniques), other model variables are monitored to ensure accurate reflection of platelet biology
Rule 3. Evaluate within context	Numerical simulations are performed under physiological and pathological shear stresses relevant to blood vessels (normal/diseased) and blood-recirculating cardiovascular devices, with appropriate blood properties (i.e. viscosity, temperature).
Rule 4. List limitations explicitly	Numerical simulations are accurate in the context of published data and in-house in vitro observations. We do not make conclusions beyond the experimentally validated conditions. Further limitations are due to capacity of the software to model biological observations and limitations of the HPC resources used.
Rule 5. Use version control	All experimental data are traced by their creation date and record the experimenters' names. All DPD-CGMD files track the creation date.
Rule 6. Document adequately	Simulation codes/model markups and changes within are tracked and shared among the simulation group. All experimental data are stored in a database (currently in video and spreadsheet format) and shared among all team members, allowing interfacing with numerical software. Protocols are shared and updated via Stony Brook's Google Drive services
Rule 7. Disseminate broadly	Simulation software and data/experimental database is currently shared via Google Drive, and we are exploring sharing broadly via the Google Cloud Platform. These items are also presented during regular meetings and national/international conferences.
Rule 8. Get independent reviews	Our algorithms and experimental data will be shared with fellow IMAG researchers with similar work (i.e. Drs. Alber and Karniadakis) for independent evaluation.
Rule 9. Test competing implementations	Within our group, we test the efficiency of various iterations of our DPD and CGMD codes to select the most appropriate model parameters (i.e. Morse potential, bond force parameters, etc.). Due to the uniqueness of our approach, we do not have an external algorithm for direct comparison.
Rule 10. Conform to standards	While there are no set standards for our platelet-based experiments, we follow commonly followed practices for blood/platelet preparation, microscopy, and statistical analysis as published in relevant experimental journals.